fjrs.futa.edu.ng

FUTA Journal of Research in Sciences

ISSN: 2315-8239

FUTA Journal of Research in Sciences, 2016 (1): 94-106

Hive management of honeybee, *Apis mellifera adansonii* Latreille and mellisopalynological and proximate analyses of honey samples from agrarian regions of Lagos State, Nigeria

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ABSTRACT

The study evaluated Integrated Hive Management (IHM) strategies that would strengthen newly established bee colonies and also identified pollen spectra as well as proximate contents of honeys from Ikorodu and Badagry areas of Lagos State, Nigeria. Hive management strategies which involved monitoring for insect pests and diseases of honeybees were put in place at the University of Lagos (Unilag), Badagry and Ikorodu apiaries. Pests encountered were recorded while preventive and therapeutic measures were implemented. Honey samples collected were analyzed for mellisopalynology and proximate contents. Adult insect pests found at the Unilagapiary were the small hive beetle, *Aethina tumida* Murray (17), the lesser Wax moth, *Achroia grisella* Fabricius (2) and Varroa mite, *Varroa destructor* Anderson and Trueman (2). Other nuisance pests such as ant species were common to all the apiaries. The result of the mellisopalynology showed that the Badagry honey samples recorded 9273 pollen grains distributed among 32 families and 51 species while the Ikorodu honey samples recorded 8564 pollen grains and spores distributed over 29 families and 46 species. The Badagry honey samples had abundance of *Nymphaea lotus, Adenia* species, *Raphia* species and *Typha* pollen. The abundance of *N. lotus* was expected due to the close proximity of the apiary to swampy vegetation. The presence of *Corchorus* and Solanaceae pollen in Ikorodu honey confirmed the closeness of a farmland to the apiary. A fern spore, cf. *Thelypteris* species was recovered from Ikorodu honey. The proximate content showed that honeys from the apiaries had water content of 19.4% and 19.8% while protein was 0.44% and 0.43% for Ikorodu and Badagry respectively.

Keywords: Integrated hive management, honeybee pests, mellisopalynology, proximate analysis

INTRODUCTION

Apiculture is the maintenance of honeybee colonies by humans for their highly desirable products and services. *Apis mellifera adansonii* Latreille, honeybees, in addition to providing products such as honey, beeswax, pollen, propolis, bee venom and royal jelly (Oyerinde *et al*., 2012); are responsible for about 80% of global agricultural pollination services (Breeze *et al*., 2011). Although, the use of honeybees for pollination services in not a common phenomenon in developing countries such as Nigeria, it however, has been reported that bees are the most important pollinators. Among them, *A. mellifera* is the primary species managed for pollination (Ricketts *et al*., 2010).

As important as honeybees are to man and the ecosystem, they are usually confronted with a lot of biotic stressors which include pests and diseases. The impacts of bee pests on colony establishment has been reported to cause about 15 % decline in honey bee colony establishment in some of the Local Government Areas of Kwara State in Nigeria (Oyerinde and Ande, 2009). Also, the behaviour of the bees such as regular absconding and aggressiveness has contributed to low colony establishment. It is usually difficult to work with the African bees because of their aggressive nature (Akinwande *et al*., 2013). It is therefore imperative to put an effective management strategy in place in order to fully exploit the benefits of honeybees. An Integrated Hive Management (IHM) programme controls pests and disease by using a combination of strategies designed to be safe, effective and economical. Many IHM practices are easy to apply and are designed to manage, but not necessarily eliminate honey bee pests. The first step in an IHM programme requires taking the time to familiarize you with the bees, the colony, and the pests. Education, monitoring, prevention, and intervention are steps on the IHM continuum. IHM intervention strategies draw from the following categories; cultural, mechanical, biological, and/or chemical controls. An effective IHM program also includes continuous evaluation and planning steps so that adjustments can be made as necessary to ensure the success of the program (Thia, 2014).

One important factor responsible for the successful establishment of colonies especially in developing countries where there are no augmented diets such as sugar syrups for bees is to identify and site apiaries at locations with diverse plants visited by bees. Pollen of various plants representing potential sources of nectar and pollen for the honey bees is an important pre- requisite for the developing apiary (Kalpana and Ramanujam, 1997). Microscopical analysis of pollen of plants forged by bees is an established method to determine the source of honey in an area. The sustainability of beekeeping is dependent on the availability of the resources required by the bees, which are predominantly nectar and pollen. Therefore, it is essential to know the plant species that contribute to maintaining

these insects (Novais and Absy, 2015). Various methods have been used to identify the plants visited by honeybees and these include direct observation of foraging bees, palynological analysis of honey, analysis of pollen loads removed from returning foragers, analysis of pollen stores in nests or hives, and secondary information on local floral resources from experienced beekeepers (Dukku, 2013). Among these, mellisopalynology gives the most reliable information on floral sources visited by honey bees. The knowledge of plants visited by bees is essential in guiding prospective beekeepers in the choice of suitable sites for locating apiaries. It is also essential in the identification of crops that may benefit from pollination by honeybees. Mellisopalynology helps to determine the contribution of each pollen type in the honey samples as well as help to detect adulteration of commercial honey. It is therefore used in regulating honey quality (Delaplane *et al*., 2013; Sorkun and Dogan, 2002).

The proximate content of honey is important in its characterization. Enzymes are present in minute amounts but have significant effect on the quality of honey and are closely related to the nutritional content and honey freshness. These enzymes break down complex sugars into simple sugars and the ratios and composition of monosaccharides and disaccharides determine the degree of honey maturity. Ripen honey usually have lower disaccharides such as sucrose and maltose content than those from honey harvested at an earlier stage. This is because most of disaccharides have been converted into monosaccharides by the action of enzymes. Hence, the predominant sugars and their ratios are crucial parameters for honey characterization.

This study was therefore conducted to monitor and evaluate some strategies of integrated hive management, identify the different pollen types and their concentrations in honey samples as well as document the results of proximate analysis of the honeys from two apiary locations in Lagos, Nigeria.

MATERIALS AND METHODS

Study area

This study was conducted at apiaries located in Badagry, Ikorodu and the University of Lagos (Unilag) Akoka Yaba in Lagos State, Nigeria. Lagos is a coastal area in southwest Nigeria located at latitude $6^0$27'N; longitude $3^0$130'E and an altitude of about 6.1 meters above sea level. The area exhibits humid climate typical of tropical environment. Lagos is characterized by two rainy seasons, with the heavy rains occurring from April to July and the light rainy season from October to November while December to March is usually dry. A short dry season is experienced in August commonly referred to as "August Break." Lagos receives an average rainfall of about 1,918 mm with

a mean monthly range of about 29 ^{o}C. The dominant vegetation of Lagos State is the swampy forest consisting of the fresh water and mangrove swamps, both of which are influenced by bi-modal rainfall pattern of the state which makes a large part of the environment a wetland.

Twenty hives were located at each of Badagry and Ikorodu areas while ten hives were located at the wetlands beside the lagoon front at the University of Lagos. Each location comprised at least five langstroth hive types while the remaining were Kenya top-bar hives.

Hive Management and Monitoring for pests of honeybees

Four colonies were inspected fortnightly for the presence of biotic stressors in hives in each location from November 2014 to October 2015. Monitoring was done mainly through visual observation from 6:00 to 9:00 am GMT before the rising of the sun when the bees were calm and the weather was clement. Before carrying out inspection, the entrance holes of the hive were lightly smoked and the lids gently removed. Thereafter, a few light puffs of smoke were administered on the bees. Inside the hives, frames, bars and combs were checked for signs of pest infestations and disease infection. Signs of sunken cells, pinholes in cappings, putrefied larvae as well as a pungent odour for American Foulbrood (AFB) while bee poop on frames or on inner surfaces of hive were observed for *Nosema apis*. The bees were examined for their colony strength and for the presence of eggs, larvae, and sealed brood. The worker bees were closely observed on their dorsal thoracic cavity and other body parts for infestation by varroa mites.

Melissopalynological analysis of honey samples

Honey samples were obtained from three hives from each of Badagry and Ikorodu apiaries. The Unilag apiary was not colonized as at the time of sample collection. Two millilitres of each sample was prepared for acetolysis . Acetolysis was carried out according to Erdtman's (1969) method with nine parts Acetic anhydride and one part concentrated Hydrogen tetra-oxo-sulphate (VI) acid (H_2SO_4). After much rinsing with water, 50 % glycerine was used to rinse further. The resulting residue was later stored in 100 % glycerine. For quantitative microscopic study, 1.0 ml of 100 % glycerine was added to all the residues. Twenty microliters each of the resulting residue and 100 % glycerine mixture was pipetted onto microslides and 'cover-slipped'. The prepared microslides were studied microscopically. Identification of recovered pollen and spores were carried through the use of standard atlases and journals (Sowunmi, 1976; Agwu and Akanbi, 1985, Gosling *et al* 2013) and the Reference Slide Collection of the Palaeobotany/Palynology Laboratory of the Department of Botany, University of Lagos. Photomicrographs of some important pollen and spore were taken and displayed in Plates 1 and 2

Determination of proximate parameters

Proximate analysis evaluated the moisture content, protein content, fat content, Ash content and Carbohydrate content. The analysis followed the procedure recommended by the Association of Official Analytical Chemists (AOAC, 1990). The protein content was determined by Kjeldahl method based on the total nitrogen content from the AOAC Official Method. The fat content was determined by using acid hydrolysis method. The dietary fibers which consisted of the total, soluble and insoluble fibers of honey samples were determined while the moisture content was measured by placing 5 g honey samples in an oven set at 105°C for 18 hours. The same samples were further analyzed for the ash content by calcinating them in a furnace (Carbolite CWF 1100, Keison Products, England) at 550 ^{o}C until constant weight following standard procedures by AOAC (1990). Carbohydrate value was estimated from the equation according to Charrondiere *et al*. (2004).

Total carbohydrate (g/100 g) = 100 – (water + ash + protein + fat + dietary fiber).

RESULTS

Hive management

The colonies appeared healthy as all stages of the bees were seen and most drone cells were found at the bottom of frame and made up of less than 20 % of cells on the frame. Ant species and reptiles such as lizards were common in all the hives and these were put in check by proper sanitation and ant infestations were stopped by greasing the stands of each hive. The apiary at Unilag recorded three different major insect pests of honeybees and these were the adults of the small hive beetle, *Aethina tumida* (17), the lesser wax moth, *Achroia grisella* (2) and varroa mite, *Varroa destructor* (2). The developing stages (eggs and larvae) of the wax moth were also observed at the Unilag apiary. There were no major insect pests or diseases of honeybees observed in Badagry and Ikorodu apiaries (Table 1). No chemical was applied to treat infested colonies; rather combs were frozen to kill all stages of the wax moth while the hive beetles and varroa mites were manually removed. There was no escalation of pests' infestation at Unilag apiary after sanitary and physical measures of control were implemented. Active colonies established were Ikorodu (75%), Badagry (55%) and Unilag (40%) as at the time of this study.

Table 1: Major pests' infestation at selected apiaries in Lagos

Pests	Unilag	Badagry	Ikorodu
Aethina tumida	*17*	*0*	*0*
Achroia grisella	*2*	*0*	*0*
Varroa destructor	*2*	*0*	*0*

Pollen spectra of honeys from Ikorodu and Badagry

Badagry apiary hives recorded 9273 pollen grains distributed among 32 families and 51 species. The Ikorodu honey samples recorded 8564 pollen grains and spores distributed over 29 families and 46 species. Diversity values in Badagry samples were higher than those from Ikorodu. It may mean that the vegetation in Badagry is richer than that of Ikorodu, probably due to vegetation loss as a result of urbanization. There was no honey flow yet in the apiary located at Unilag as at the time of this study.

The Badagry honey samples had abundance of *Nymphaea lotus* and *cf. Drepanocarpus, Raphia* spp. and *Typha* pollen (Table 2).

Table 2: Palynomorphs recovered from Badagry honey samples

Palynomorphs	**Family**	**Percentage Abundance (%)**		
		Hive$_1$	**Hive$_2$**	**Hive$_3$**
Acacia	Mimosaceae	-	0.02	-
Acanthaceae	Acanthaceae	0.05	-	-
Adenia cisssampeloides	Passifloraceae	3.96	0.11	0.85
Albizia zygia	Mimosaceae	-	-	0.07
Alchornea	Euphorbiaceae	0.16	0.13	0.68
Amaranthaceae	Amaranthaceae	1.10	0.02	-
Arecaceae	Arecaceae	-	0.09	0.47
Asteraceae	Asteraceae	1.10	2.55	2.37
Asystasia gangetica	Acanthaceae	-	0.02	-
Borreria sp.	Rubiaceae	-	-	0.03
Cammiphora africana	Burseraceae	-	-	0.03
Celtis sp.	Cannabaceae	-	-	0.07

cf. Bridelia	Euphorbiaceae	-	0.02	-
cf. Chromolaena odorata	Asteraceae	-	-	0.20
cf. Chrysophyllum sp.	Sapotaceae	0.60	0.07	0.34
cf. Corchorus sp.	Tiliaceae	-	-	0.10
cf. Dodonea viscosa	Euphorbiaceae	0.11	-	-
cf. Morinda lucida	Rubiaceae	-	-	0.03
Charred Poaceae Epidermis		-	-	0.07
Citrus sp.	Rutaceae	-	-	0.07
Cleistopholis patens	Annonaceae	-	0.04	0.10
Cocos nucifera	Arecaceae	-	-	0.07
Combretum sp.	Combretaceae	0.55	0.02	0.14
Crudia sp.	Caesalpinaceae	0.49	-	0.44
Cyperus sp.	Cyperaceae	0.05	-	0.10
cf. Drepanocarpus sp.		57.94	19.91	25.80
Elaeis guineensis	Arecaceae	1.10	0.56	0.81
Grewia sp.	Tiliaceae	3.30	-	-
Heliotropium sp.	Boraginaceae	-	0.02	-
Hyptis sp.	Lamiaceae	-	-	0.14
Indet. Pollen		0.60	0.07	0.54
Ipomoea sp.	Convolvulaceae	-	-	0.17
Jasminum sp.	Oleaceae	-	0.02	-
Malvaceae	Malvaceae	-	0.02	0.14
Menispermaceae	Menispermaceae	-	0.02	-
Nymphaea lotus	Nymphaeaceae	16.27	34.37	43.53
Ocimum basilicum	Lamiaceae	-	-	0.07
Papilionaceae	Papilionaceae	0.38	0.07	0.37
Poaceae	Poaceae	-	0.02	0.24
Polygonium sp.	Polygonaceae	-	0.02	0.20
Psidium guajava	Myrtaceae	0.55	-	0.37
Psilatricolporate pollen		6.60	1.97	2.91

Raphia spp.	Arecaceae	0.22	29.07	1.76
Retitricolpate pollen		0.55	0.22	-
Sida	Malvaceae	0.05	-	-
Solanum sp.	Solanaceae	0.05	0.02	-
Symphonia globulifera	Clusiaceae	-	-	0.07
Syzygium guineense	Myrtaceae	0.11	-	0.03
Tilia	Tiliaceae	-	0.20	0.20
Typha sp.	Typhaceae	3.85	10.20	15.57
Uapaca acuminata	Euphorbiaceae	0.05	0.09	0.81
Umbelliferae	Umbelliferae	-	-	0.03
Vernonia sp.	Asteraceae	0.16	-	-

Each hive samples exhibited its own unique diversity and assemblage, though there are some similarities to indicate the hives' closeness to one another. The abundance of *N. lotus* was expected as the apiaries were close to swampy vegetation. *Typha*, a popular grass-like plant around the area, is also of appreciable quantity in the Badagry apiary hives. This pollen grain may have been used as food.

The Ikorodu honey samples recorded abundance of *Adenia cissampeloides, Nymphaea lotus* and Amaranth pollen grains (Table 3). The presence of *Corchorus* and Solanaceae pollen is most likely indicating that farmland is close to the apiary. This is the first time *Adenia cissampeloides* is recovered in such high proportions from honey samples in Nigeria. Both locations exhibited pollen assemblages representing their vegetation types.

Table 3: Palynomorphs recovered from Ikorodu honey samples

Palynomorphs	**Family**	**Percentage Abundance (%)**		
		Hive$_1$	**Hive$_2$**	**Hive$_3$**
Adenia	Passifloraceae	74.8	67.05	87.87
Alchornea cordifolia	Euphorbiaceae	-	3.40	0.26
Allophylus africanus	Sapindaceae	-	-	0.04
Amaranthaceae	Amaranthaceae	2.3	14.00	1.66
Arecaceae	Arecaceae	0.23	-	-

Asteraceae	Asteraceae	1.84	0.91	0.79
Balanites sp.		-	0.02	0.23
cf. Acanthaceae	Acanthaceae	-	-	0.15
cf. Entada abyssinica	Mimosaceae	-	0.12	0.15
cf. Pandanus	Pandanaceae	-	-	0.04
cf. Thelypteris spore	Polypodiaceae	-	0.56	1.02
Chrysophyllum sp.	Sapotaceae	-	0.09	-
Cleome	Cleomaceae	-	0.05	-
Combretum	Combretaceae	0.23	0.05	-
Corchorus olitorius	Tiliaceae	-	0.47	-
Cordia cf. *vignei*	Boraginaceae	0.12	0.12	0.15
Crudia	Caesalpinaceae	1.04	0.59	0.38
Cyperus	Cyperaceae	0.23	0.09	0.19
cf. Drepanocarpus sp.		3.8	2.09	2.34
Elaeis guineensis	Arecaceae	1.27	0.98	0.19
Epidermal cells		-	-	0.04
Euphorbia	Euphorbiaceae	-	-	0.19
Fungal spore	Fungi	-	0.05	-
Grewia	Malvaceae	0.12	-	-
Hippocratea	Hippocrateaceae	-	-	0.04
Indet. Pollen		1.15	0.26	0.15
Ipomoea	Convolvulaceae	0.12	-	0.04
Khaya	Meliaceae	-	0.05	-
Luffa	Cucurbitaceae	-	-	0.11
Mimosa pudica	Mimosaceae	0.23	0.12	-
Mussaenda sp.	Rubiaceae	-	0.33	-
Nymphaea lotus	Nymphaeaceae	5.18	4.57	2.76
Papilionaceae	Papilionaceae	1.5	0.02	-
Phyllanthus	Euphorbiaceae	1.04	-	-
Poaceae	Poaceae	-	0.07	0.45

Polygonum	Polygonaceae	-	0.07	-
Psidium guajava	Myrtaceae	1.04	0.38	0.08
Psilatricolporate pollen (Small)		-	0.68	0.04
Raphia	Arecaceae	0.12	0.12	-
Retistephanoporate pollen		0.12	-	-
Retitricolporate pollen		0.35	-	-
Senna sp.	Caesalpinaceae	1.38	0.12	0.11
Solanum cf. *nigrum* (pepper)	Solanaceae	-	1.55	0.11
Syzygium guineense	Myrtaceae	-	0.12	0.04
Typha	Typhaceae	0.23	0.40	0.30
Umbelliferae	Umbelliferae	0.12	0.12	0.08
Verrucate spore	Ferns	1.5	-	-

Proximate analysis of honey samples

The results of proximate analysis for all honey samples are presented in Table 4. The moisture content of honey samples from Badagry and Ikorodu apiaries were 19.8 and 19.4 % while the protein content were 0.43 and 0.44 %, respectively. This signified that the protein content of the honeys were 43mg and 44mg in 100g of honey. The carbohydrate content of honeys from Badagry and Ikorodu were 75.65 and 76.41, respectively (Table 4).

Table 4: Proximate analyses of honeys from Ikorodu and Badagry apiaries

Apiary	Moisture content (%)	Ash content (%)	Protein content (%)	Fat content (%)	Carbohydrate content (%)
Ikorodu	19.4	0.64	0.44	0.09	76.41
Badagry	19.8	0.67	0.43	0.09	75.65

DISCUSSION

Diseases, pests and parasites are biotic factors while environmental factors and pesticide poisoning are abiotic factors that pose a great threat to beekeeping. In Nigeria, beekeepers are oblivious of the effect of biotic stressors in their hives and

hence, do not have any IHM strategy to mitigate pests problems when present. Of the three apiaries evaluated for pests' infestations, only Unilag apiary recorded the presence of major hive pests. This may not be unconnected with the challenges encountered during colony establishment at Unilag. During establishment at Unilag, baited hives were unable to attract honeybees; therefore, bee colonies were obtained from an existing apiary in Osun State, Nigeria. This therefore underscores the fact that exchange of hive equipment as well as buying colonies from other location has a tremendous tendency in spreading beehive pests, parasites and diseases. The presence of these pests at Unilag apiary affected honey production. This study is in agreement with the assertion of Akinwande (2013) who reported that there were annual declines of 3.43 %, 5.46 % and 4.93 %, in colony establishment in Lagos, Ogun and Osun States, respectively due to biotic stressors of honeybees. Most of the beekeepers do not manage their colonies properly because of the widely held opinion that local top bar hives do not require constant inspection and also due to the aggressiveness of the bees. However, observed presence of pests and diseases, poor hive and seasonal management, ecological problems and lack of queen rearing capacities were potential problems raised by the beekeepers.

Integrated Pest Management (IPM) is a strategy aimed at discontinuing prophylactic treatments of miticides through the judicious use of pest monitoring methods, economic thresholds, and management tactics so that miticide applications targeting Varroa mites are only performed when necessary (Delaplane *et al*., 2005). In Nigeria, miticides are not applied and so it becomes imperative to educate and train local beekeepers on the need for regular monitoring and implementation of cultural control practices that will drastically reduce the chances of their apiaries being infested by pests and parasites.

The honey samples have given complete information on the botanical and geographical origin. This is in consonance with earlier reports of the botanical and ecological origins of honey samples, the honey plants and honey quality as documented by Sowunmi (1976), Agwu and Alkanbi (1985), Adeonipekun (1989). Adekanmbi and Ogundipe (2009), Adeonipekun (2010) and Aina *et al.* (2015) across the vegetation zones in Nigeria. However, the recovery of a fern – cf. *Thelypteris* species in Ikorodu was very unusual. Pteridophytes are not known to be used by bees for honey making and for food. They lack all the attractive adaptations including the hidden position of their sori that contain the sporangia housing the spores. Their recovery in reasonable proportions from the Ikorodu hives may indicate that this particular fern has its sori probably more brightly coloured, and the spores being very nutritious. It could also be that the fern may be a climber or an

epiphyte on a particular tree foraged by the bees. This is therefore the first time to the best knowledge of the authors, that a fern spore is recovered in this magnitude from any Nigerian honey samples.

Proximate analysis is usually carried out to determine the nutritional values of foods and food based products. The nutrient content is essential not only for health promotion, but also for metabolic energy. All honey samples were considered to be genuine because their total nitrogen contents were more than 50 mg per 100 g of honey, which exceeded the limit of 30 mg per 100 g of honey (Rodriguez *et al*., 2004). Though, the honey samples had moisture content of 19.8 and 19.4 %, it is very important to have the moisture content further reduced so that the honey samples are not prone to granulation and fermentation due to high moisture content. Moisture content of honey higher than 20 % is considered high (Rodriguez *et al*., 2004). From the result, the fat content is very low and this is in agreement with that reported by other authors (Alvarez-Suarez *et al*., 2010). High fat content makes foods to be susceptible to rancid spoilage during storage.

ACKNOWLEDGEMENTS

We are grateful to the Lagos State Research and Development Council (LRDC) under the visionary leadership of the immediate past Governor of the State, His excellency, Mr. Babatunde Raji Fashola, for funding this work. The State government for the first time funded over 30 different projects of which this study was among. We are also grateful to the management of the University of Lagos for the supervisory role played in ensuring the success of this project.

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